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Biological Activity of a Furanyl Anti-Juvenile Hormonal Compound on Triatomine Larvae

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Abstract: Applied topically to larvae of *Rhodnius prolixus, Triatoma infestans, Triatoma Vitticeps, Panstrongylus herrera* and *Panstrongylus megistus* a novel, synthetic, furan-containing anti-juvenile hormonal compound, 2-(2-ethoxyethoxy)ethyl furfuryl ether (EEFE) induced a variety of biological responses attributable to an enforced cessation of juvenile hormone secretion, including precocious metamorphosis into diminutive adultoids and retardation of molting. The highest percentage of precocious adultoids was obtained when EEFE was applied prior to feeding. In decreasing order of sensitivity to this anti-juvenile hormone (AJH) were *T. infestans, R. prolixus, P. herrera, P. megistus* and *T. vitticeps*. The ED_{50} of EEFE in *R. prolixus* compared well with that of the botanically derived AJH, precocene II, but *T. infestans* was significantly more sensitive to the furanyl antihormone than to precocene II. The significance of these findings is discussed in terms of the complexity of expression of the anti-juvenile hormonal activity during the post-embryonic development of *triatomines*.

Key words: *Rhodnius, Triatoma, Panstrongylus, Triatomines,* anti-juvenile hormone, precocious metamorphosis, adultoid.

1 INTRODUCTION

Beginning with the early studies[1,2] of the precocenes, it has been evident that compounds with anti-juvenile hormonal (AJH) activity for insects must be considered among the novel, biorational approaches to insect control. AJH activity, following the induced cessation of juvenile hormone secretion, causes the developing immature stages to discontinue their juvenile progression of development and to undergo premature metamorphosis into diminutive, sterile adults.[1–6] Following the discovery of the AJH precocenes in *Ageratum* sp. additional phytochemicals were discovered to possess AJH activity in insects.[7,8] Although several AJHs have been produced by synthetic chemical approaches,[9–16] none possessed sufficient activity against important pest species to warrant commercial development. A series of novel furanyl ethers were recently demonstrated to possess potent AJH activity in the milkweedbug, *Oncopeltus fasciatus* Dall.[17] This study examines the activity of EEFE, one of the most active of the furanyl ether AJHs, on the hematophagous insect *Rhodnius prolixus* Stahl. Observations include the induction of precocious metamorphosis, prolongation of molting, and lethality under different modes of treatment. In addition we have investigated the sensitivity of EEFE on four additional species of *Triatomines*.

2 MATERIALS AND METHODS

2.1 Insects

Third-instar larvae of *R. prolixus, Triatoma infestans* Klug, *T. vitticeps, Panstrongylus megistus* (Burm.) and

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P. herrera (donated by Dr J. Jurberg, Dept of Entomology, FIOCRUZ) were used throughout this investigation. Larval weights just before feeding ranged from 4·5 mg for *R. prolixus* to 7·6 mg for *P. herrera*. Insects were maintained at 28°C throughout the experiments.

2.2 EEFE treatments

EEFE and precocene II were synthesized as described previously.[1,17] Their purity was estimated as >97% by gas chromatography. Following ecdysis, third-instar larvae of the five species of *Triatomines* were starved for 30–35 days and then fed on citrated mouse blood (to each 100 ml of mouse blood was added 1 ml of anticoagulant solution composed of 18·4 g glucose, 16·5 g sodium citrate, 6·0 g citric acid, dissolved in redistilled water to a final volume of 100 ml). Oral treatments with EEFE were performed only on third-instar larvae of *R. prolixus* by adding the test compound to the blood meal at concentrations of 15, 30 and 60 $\mu g\ ml^{-1}$ blood. The continuous contact method of treatment was as described previously.[1] EEFE, dissolved in acetone, was applied evenly to the bottom of a 9-cm Petri dish at dosages ranging from 0·05 to 0·5 mg. Following solvent evaporation, a residue of the compound remained on the bottom surface (area = 61 cm^2) of the Petri dish, yielding surface residues of 0·82–8·2 $\mu g\ cm^{-2}$. Twenty third-instar larvae of *R. prolixus* were placed on the treated surface and the Petri dish cover replaced. Insects were removed from the Petri dish only for feeding three days after beginning the treatment and returned to the Petri dish until the end of the experiment (25 days after feeding). Petri dishes containing insects were held at 28°C throughout the experiment.

Topical treatments were performed on groups of 20–30 third-instar larvae of *R. prolixus*, *T. infestans*, *T. vitticeps*, *P. megistus* and *P. herrera*. EEFE at concentrations of 15–60 μg in 0·5 μl of acetone was applied to the ventral surface of the abdomen on the fourth day before feeding unless otherwise stated. Larvae of all five species always experienced a high percentage of molting if well fed in one feeding session. Only fully engorged insects were used in all treatments. Controls received solvent treatments only. Feeding, continuous contact, topical treatments and control experiments were repeated three times for each species studied.

2.3 Biological evaluations

The AJH activity of EEFE was determined by the induction of precocious metamorphosis in the five species of *Triatomines* studied. In addition, other biological activities observed and recorded were molting retardation, molting stasis and toxicity (i.e. 24-h mortality). These data were accumulated through daily examination of all insect groups. The period of observation was 30 days, which accommodated the maximum molting period of the control groups except for *R. prolixus* in which the observations were carried out for only 22 days.

2.4 Comparison of EEFE and precocene II for effective doses (ED_{50}) to induce adultoid formation

In this experiment only third-instar larvae of *R. prolixus* and *T. infestans* were used, in groups of 30 insects, and the effects of EEFE and precocene II compared. Each of the *triatomine* species was treated topically with 10, 25, 50 or 100 μg of EEFE or precocene II. These experiments were repeated twice. Doses required for induction of precocious metamorphosis in 50% of the treated insects (ED_{50}) were determined by regression analysis of the doses used and probits of percentages of surviving insects which molted to premature adults.

3 RESULTS AND DISCUSSION

3.1 Continuous contact and feeding treatments with *Rhodnius prolixus*

Oral and continuous contact treatments had no apparent effects either on precocious metamorphosis or on the duration of the intermolt period in comparison with controls, even when the insects were fed treated blood again and observed until the next ecdysis. Oral treatment caused low mortality (less than 20%) up to the highest concentration of 60 $\mu g\ ml^{-1}$. However, continuous contact treatment caused more than 50% mortality when dosages exceeded 2·0 $\mu g\ cm^{-2}$ (Table 1).

3.2 Topical treatment of *Rhodnius prolixus*

Pilot experiments demonstrated significant sensitivity to topical applications with EEFE in *R. prolixus*. There-

TABLE 1
Effects of Continuous Contact and Oral Treatments of 2-(2-Ethoxyethoxy)Ethyl Furfuryl Ether (EEFE) on Third-Instar Larvae of *Rhodnius prolixus*

Treatment	*Dosage*[a]	*Mortality* (%)	*Precocious adultoids* (%)
	—	12	0
Continuous	0·82	17	0
contact	2·0	28	0
($\mu g\ cm^{-2}$)	3·0	55	0
	4·1	80	0
	8·2	88	0
	—	7	0
Oral	10	8	0
($\mu g\ ml^{-1}$ blood)	30	12	0
	60	19	0

[a] Groups of 20–30 insects. Each datum represents the mean of three experiments.

fore, topical treatments seemed the mode of choice with which to identify the developmental period most sensitive to the anti-hormonal action. Carefully staged third-instar larvae were given a series of topical applications of 60 μg per insect before and after a blood meal. The most critical period of sensitivity was established by observing the point in time after EEFE treatment at which the production of adultoids increased. Adultoid induction was maximum when EEFE was applied from day 6 to day 3 before feeding. However, treatment with 60 μg per insect EEFE immediately post feeding and until three days after feeding produced less than 5% precocious adultoids but caused significant (over 70%) mortality.

Studies were concentrated on the effect of different AJH dosages on mortality, ecdysial stasis and induction of precocious metamorphosis following treatment of *R. prolixus* larvae four days before feeding. Table 2 shows low mortality by topical treatment with 30 μg (12%) and 60 μg per insect (18%) compared with controls (10%). Whereas complete ecdysis in controls occurred within 10–14 days, treated insects experienced retarded molting until 16–22 days after feeding. Furthermore, the treatment with EEFE resulted in 70% and 55% molting following treatment with 30 and 60 μg per insect, respectively, within days 10–14 post treatment, whereas control molting was complete (100%) within this period. However, all treated insects eventually molted by the end of the experiment (22 days post feeding) (Table 2). Although the treatment of 15 μg per insect had no apparent effect on ecdysis, lethality of adultoid formation, dosages of 30 and 60 μg per insect induced 15% and 50%, respectively, of the larvae to molt into adultoids (Table 2).

Visual inspection of adultoids revealed that topical treatments with EEFE produced *Rhodnius* adultoids with fully adult abdominal cuticle, ocelli and tibial adhesive organs as well as rudimentary adultoid wings possessing full articulation with the thorax. Nevertheless, the precocious adults were short-lived, and did not feed. Adultoids sometimes died during ecdysis and were confined within the old cuticle. Similar morphological characteristics have previously been observed in adultoids of *R. prolixus* treated with other allatotoxins, including precocenes and isopentenylphenols.[7,18]

3.3 Topical treatment in other *triatomine* species

The AJH effects of EEFE on mortality, molting delay and precocious metamorphosis were also examined by topical treatment of third-instar larvae of *T. infestans*, *T. vitticeps*, *P. herrera* and *P. megistus*. Table 2 shows that EEFE was relatively non-toxic (less than 20%) to *P. megistus*, *P. herrera*, *T. vitticeps* and *T. infestans*, yielding results similar to those for *Rhodnius*. However, treatment with EEFE induced significant delays in ecdysis compared to that of controls, which began on day 10 post feeding and was well advanced by day 14

TABLE 2

Induction of Precocious Metamorphosis, Ecdysial Stasis and Mortality following Topical Treatment of Third-Instar larvae of Several Species of Triatomines with 2-(2-Ethoxyethoxy)Ethyl Furfuryl Ether (EEFE) Four Days before Feeding

Species	*Dose*[a] (*μg per insect*)	*Molting Day 10–14* (%)	*Total molting* (%)	*Mortality* (%)	*Precocious adultoids* (%)
R. prolixus	—	100	100	10	0
	15	95	100	5	0
	30	70	100	12	15
	60	55	100	18	50
T. infestans	—	50	72	6	0
	30	25	65	7	60
	60	15	71	9	90
T. vitticeps	—	30	50	5	0
	60	15	65	3	10
P. herrera	—	45	55	5	0
	60	25	60	2	50
P. megistus	—	30	65	8	0
	30	10	45	10	15
	60	0	54	18	25

[a] Groups of 20–30 third-instar larvae were treated topically with 0·5 μl acetone, or 0·5 μl acetone containing 15, 30 or 60 μg of EEFE. Each datum represents the mean of three experiments.

(Table 2). For example, during the first four days of molting of the controls of *T. infestans* larvae (e.g. day 10–14 post feeding) one half (50%) had molted while only 25% and 15% of *T. infestans* treated respectively with 30 μg and 60 μg of EEFE were able to molt. Similar molting delays were observed in the other *triatomine* species during the first four days of molting that saw completion of molting by the controls (Table 2). However, with the exception of *Rhodnius*, the total percentage of ecdysis for all controls never attained more than 72% during the entire experimental period of observation (i.e. 22 days). While EEFE induced precocious metamorphosis in all groups of *triatomine* species treated, *T. infestans* was clearly the most sensitive to the antihormone. Although dosage-dependent differences were observed in the percentage of induced precocious metamorphosis, the precocious adultoids produced in all species of *triatomines* were morphologically similar to those described above for *R. prolixus*.

3.4 Comparison of the ED_{50} required for adultoid formation by EEFE and precocene II

Since the mode of action, species specificity and spectrum of activity of the furanyl compounds are presently unknown[17] it seemed valuable to compare the AJH activity of EEFE with that of the precocenes, whose mode of action is understood to involve alkylation of nucleophilic substrates following activation by oxidation. In this experiment we compared the relative AJH activity of the EEFE with that of precocene II by topical treatment of third-instar *R. prolixus* and *T. infestans* larvae. Table 3 shows that the ED_{50} values for both compounds against *Rhodnius* were similar, requiring 40 and 55 μg per insect for precocene II and EEFE respectively. However, we also compared the ED_{50} values of EEFE and precocene II on *Triatoma* larvae and EEFE was found to be more active than precocene II. In this case the ED_{50} for EEFE was 28 μg per insect compared to an ED_{50} for precocene II of 180 μg per insect, a more than 6-fold difference. It appears that, among the *triatomines*, the sensitivity to EEFE and precocene II is remarkably species-specific.

TABLE 3

Comparison of the Effective Doses (ED_{50}) of EEFE and Precocene II required for Induction of Precocious Metamorphosis in Third-Instar Larvae of *Rhodnius prolixus* and *Triatoma infestans* when applied Topically Four Days prior to Feeding

Triatomine[a]	*Compound tested*	ED_{50} (*μg per insect*)
R. prolixus	EEFE	55
	Precocene II	40
T. infestans	EEFE	28
	Precocene II	180

[a] Groups of 30 third-instar larvae of each species received topical treatments of 0·5 μl of acetone or acetone containing EEFE. Each experiment was repeated twice.

3.5 General discussion

Of the 21 furanyl compounds previously reported to possess AJH activity against the milkweedbug[17] we investigated only one of the most active analogs, 2-(2-ethoxyethoxy)ethyl furfuryl ether (EEFE), in the present study. Given the paucity of information regarding its activity spectrum we tested the furan derivative against five species of hematophagous insects which are the principal vectors of *Trypanosoma cruzi*, the causative agent of *Chagas'* disease. Topical application of the AJH induced varying percentages of premature metamorphosis in all of the *triatomines*, but resulted in relatively low toxicity. However, EEFE was ineffective when given orally even up to doses of 60 μg ml^{-1} in the blood meal or by continuous contact treatment up to doses of 8·2 μg cm^{-2} in contrast to the precocenes, which have been shown previously to be highly active in these *triatomines* by both routes of administration.[7,18,19] The ineffectiveness of EEFE *per os* suggests that it is more effectively metabolized in the gut than are the precocenes. In addition to the induction of precocious adultoids, EEFE also retarded molting, but did not induce the permanent ecdysial stasis which has been demonstrated previously in these insects with the precocenes.[19] Currently, it is unknown whether the retardation of molting is due to an effect on the corpus allatum or on other target glands or tissues. Allatectomy or precocene treatment of *Rhodnius* larvae also induces retardation of molting as well as ecdysial stasis, and these treatments are well correlated with the absence of juvenile hormone and to ecdysteroid-dependent development in this insect.[18,20,21] Delayed molting in *Rhodnius* has been shown to be due to direct effects of precocene on the prothoracic glands causing a decreasing release of the ecdysteroids required for ecdysis.[7,19]

The AJH activity of EEFE was maximal in inducing premature metamorphosis with minimal toxicity if the compound was applied topically before feeding, but treatment with EEFE just after feeding caused high mortality. Without a clear understanding of the mode of action of EEFE it was impossible to determine the timing of greatest sensitivity to it except by empirical testing. It has been demonstrated that the ability of precocene to induce precocious metamorphosis in the milkweed bug is related to the part of the molting cycle during which juvenile hormone is being synthesized.[4,22] Nevertheless, the administration of precocene to *R. prolixus* larvae prior to feeding, during a time when

juvenile hormone biosynthesis is expected to be essentially quiescent, will also induce adultoid formation.[23] Possibly the corpus allatum of *R. prolixus* remains sensitive to precocene or EEFE, irrespective of whether it is undergoing active biosynthesis of juvenile hormone.

It appears that the sensitivity of insects to AJH agents is quite species-specific. Earlier studies compared the relative activities of furanyl AJHs and precocene II to induce premature metamorphosis in *Oncopeltus*[17] and concluded that EEFE was nearly twice (i.e. 1·8 ×) as active as precocene II. However, the present studies reveal considerable variation in sensitivity among species. For example, *T. infestans* was more sensitive to EEFE than to precocene II, while *R. prolixus* demonstrated about equivalent sensitivity to both AJHs (Table 3). A study of the dynamics of cuticular penetration, pharmacokinetics and metabolism/detoxification will be necessary to clarify the factors underlying the variation in sensitivity to AJHs.

While the mechanism of action of the furanyl AJHs in insects is unknown, the precocenes are clearly allatal cytotoxins, since the corpora allata are entirely destroyed.[1,2,24,25] Although it is not clear whether EEFE directly affects the corpus allatum, it must terminate juvenile hormone biosynthesis/secretion rather than interfere or compete at a receptor site, since precocious metamorphosis is prevented by exogenous treatment with juvenile hormone.[17] Notwithstanding consideration of their mode of action, our studies show potency against insects of economic and public health importance comparable with other AJHs and suggest that the furanyl ring derivatives be investigated more widely for their potential as insect control agents (for review see Ref. 16).

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